

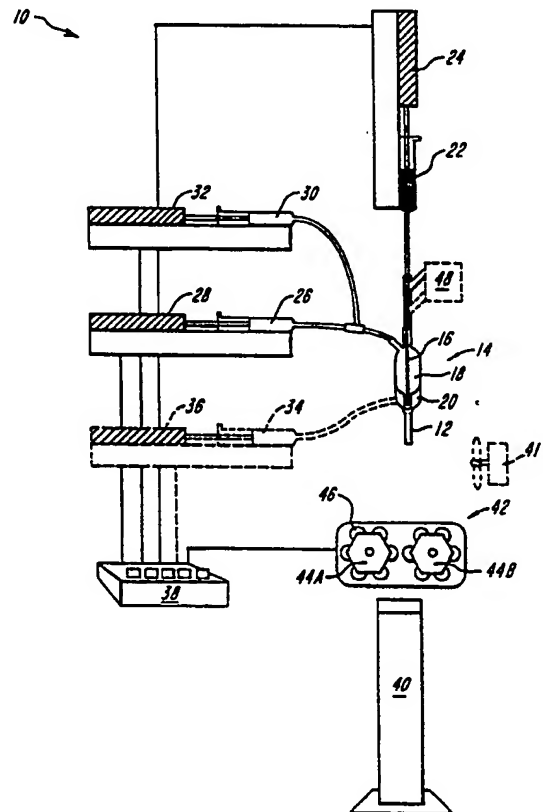


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(54) Title: CELL CAPSULE EXTRUSION SYSTEMS**(57) Abstract**

Living cells (56) which produce biologically active factors can be encapsulated within a semipermeable, polymeric membrane by co-extruding an aqueous cell suspension (54) and a polymeric solution (52) through a common port (14) to form a tubular extrudate (12) having a polymeric membrane which encapsulates the cell suspension. For example, the cell suspension and the polymeric solution can be extruded through a common extrusion port having at least two concentric bores (16 and 18), such that the cell suspension is extruded through the inner bore (16) and the polymeric solution is extruded through the outer bore (18). The polymeric solution coagulates to form an outer coating or membrane. As the outer membrane is formed, the ends of the tubular extrudate can be sealed to form a cell capsule. In one embodiment, the tubular extrudate is sealed at intervals to define separate cell compartments connected by polymeric links (58). In another embodiment, a cell capsule connected to a tethering filament (59) can be formed.



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CELL CAPSULE EXTRUSION SYSTEMS5 Background of the Invention

The technical field of this invention concerns the encapsulation of living cells for the production of biologically active factors.

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There is considerable interest at present in the biologically active products of living cells, including, for example, neurotransmitters, hormones, cytokines, nerve growth factors, angiogenesis factors, blood coagulation factors, lymphokines, enzymes and other therapeutic agents. There is also substantial interest in developing new methods and systems for producing such biological factors as well as in delivering these factors to subjects for therapeutic purposes.

20

For example, Parkinson's disease is characterized by the degeneration of the dopaminergic nigrostriatal system. Striatal implantation of polymer rods which release sustained amounts of a neurotransmitter, dopamine, has been reported to alleviate experimental Parkinsonism in rodents, indicating that the release of dopamine alone in the proper target structure may be able to correct this functional deficiency.

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In contrast to the limited capacity of a polymeric matrix drug release system, encapsulated dopamine-releasing cells have been proposed as a means to provide a continuous supply of
5 neurotransmitters. The encapsulation of neurotransmitter-secreting cells by a permselective membrane which permits diffusion of the biological factor may not only prohibit the escape of mitotically active cells, but also prevent host
10 rejection in the case of cross-species transplantation.

A number of researchers have proposed the use of microcapsules -- tiny spheres which
15 encapsulate a microscopic droplet of a cell solution -- for both therapeutic implantation purposes and large scale production of biological products. However, there are a number of shortcomings to the microencapsulation approach: the microcapsules can be
20 extremely difficult to handle (and retrieve, after implantation); their volume is limited; and the types of encapsulating materials which can be used are constrained (by the formation process) to polymers which can dissolve in biocompatible solvents.

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An alternative approach has been macroencapsulation, which typically involves loading cells into hollow fibers and then closing the extremities at both ends with a polymer glue. In contrast to microcapsules, macrocapsules offer the advantage of easy retrievability, an important feature in therapeutic (especially, neural) implants. However, the construction of macrocapsules in the past has often been tedious and labor intensive. Moreover, due to unreliable closure, conventional methods of macroencapsulation have provided inconsistent results.

There exists a need for better techniques for macroencapsulation of cells for both therapeutic implantation and industrial production purposes. Encapsulation techniques which can be practiced in a an automated fashion, and which permit the usage of a wider range of materials and/or provide more reliable closure would satisfy a long felt need in the art.

Summary of the Invention

Methods and systems are disclosed for encapsulating viable cells which produce
5 biologically-active factors. The cells are encapsulated within a semipermeable, polymeric membrane by co-extruding an aqueous cell suspension and a polymeric solution through a common port to form a tubular extrudate having a polymeric outer
10 coating which encapsulates the cell suspension.

In one aspect of the invention, methods are disclosed in which the cell suspension and the polymeric solution are extruded through a common
15 extrusion port having at least two concentric bores, such that the cell suspension is extruded through the inner bore and the polymeric solution is extruded through the outer bore. The polymeric solution coagulates to form an outer coating. As the outer
20 coating is formed, the ends of the tubular extrudate can be sealed to form a cell capsule. In one illustrated embodiment, the tubular extrudate is sealed at intervals to define separate cell compartments connected by polymeric links.
25

Strings of cell capsules formed in this manner have a number of advantages over conventional, cell-encapsulating products. The multi-compartment form ensures that breaks in the tubular membrane can
30 be contained to individual cell capsules. Moreover, the design is particularly advantageous in preparing implantable cell cultures for delivery of

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biologically-active factors to a subject for therapeutic purposes. The string of cell capsules can be coiled, twisted or otherwise deposited in various shapes to provide a dense and compact
5 structure for implantation. Because the cell capsules are connected to each other, they can also be readily retrieved, if necessary, following implantation. The string-like nature of these products is particularly preferable over individual
10 spherical microcapsules which typically are retrieved by aspiration (often resulting in a high percentage of unretrievable capsules and, consequently, inflammation in the subject).

15 Multi-compartment cell capsule strings can be formed from the tubular extrudate of the present invention by sealing the extrudate at intervals using various techniques. For example, the extrudate can be sealed by compressing it at intervals using
20 mechanical or pneumatic force. Alternatively, the pressure under which the cell suspension or the polymeric solution is extruded can be modified to collapse the tubular extrudate at intervals and define separate cell compartments. In yet another
25 technique, the flow of the cell suspension can be interrupted or otherwise impeded at intervals to likewise collapse the tubular extrudate and define cell compartments.

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The products of the present invention are particularly well-suited for use and therapeutic implant devices, such as those disclosed in U.S. Patent 4,892,538, "In Vivo Delivery Of

5 Neurotransmitters By Implanted, Encapsulated Cells" by Aebischer et al. issued January 9, 1990, herein incorporated by reference. In U.S. Patent 4,892,538, techniques are disclosed for implanting encapsulated neurotransmitter-secreting cells into a target region

10 within a subject's brain, such that the encapsulated cells secrete a neurotransmitter and thereby permit constitutive delivery of a therapeutic agent to treat a neurological deficiency, such as Parkinson's disease. Alternatively, artificial organs capable of

15 secreting other biological factors, such as hormones (e.g., insulin, thymic factors and the like) can also be constructed using the tubular extrudates and multi-compartment cell capsule strings of the present invention.

20

The cell capsules are also well-suited for use in bioreactors and other in vitro culturing systems, for the production of drugs and other useful biological materials. In such applications, cells

25 which produce such materials, either naturally, by mutation or by recombinant design, are encapsulated and allowed to synthesize the materials which can be collected following secretion into a circulating culture medium. Alternatively, the biological

30 materials can be accumulated within the cell capsules (e.g., by appropriate control of the porosity) and then harvested by removing the strands from the culture medium, lyzing the polymeric membranes and recovering the synthesized materials in concentrated

35 form.

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The polymeric coating is preferably a semipermeable membrane, that is to say, a porous structure capable of protecting transplanted cells from autoimmune or viral assault, as well as from
5 other detrimental agents in the external environment, while allowing essential nutrients, cellular waste products and cell secretions to diffuse therethrough. As used herein, the term "selectively permeable" or "semipermeable" is used to describe
10 biocompatible membranes which allow diffusion therethrough of solutes having a molecular weight up to about 150,000 (Mr).

The permeability of the polymeric coating
15 can be varied by controlling the viscosity of the polymeric solution, such that upon coagulation, the coating will form with a network of microchannels to provide diffusion pathways. In one embodiment, this can be achieved by employing a water-miscible solvent
20 as a component of the polymeric solution and maintaining a pressure differential between the aqueous cell suspension and the polymeric solution during extrusion. As the tubular extrudate forms, water from the aqueous cell suspension infiltrates
25 into the coagulating polymer to replace the solvent as the solvent is driven outward by the pressure difference. Upon coagulation, the water which has infiltrated into the polymeric coating provides a network of pores. The optimal pressure and viscosity
30 will, of course, vary with the solvent and polymer employed but can be readily ascertained for any particular polymer/solvent combination by those skilled in the art without undue experimentation.

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In another aspect of the invention, systems are disclosed for encapsulating cells to produce the tubular extrudate and multi-compartment cell capsule products described above. This system can include an
5 extrusion head assembly (e.g., a spinneret or the like) having a first inner bore and a second, concentric, outer bore, as well as a cell suspension supply means for supplying the aqueous cell
suspension to the inner bore of the extrusion head
10 assembly, and a polymeric solution supply means for supplying the polymeric solution to the outer pore of the extrusion head assembly. As the cell suspension and polymeric solution are co-extruded, they form a tubular extrudate having a polymeric outer coating
15 which encapsulate the cell suspension.

The tubular extrudate can be sealed at intervals by any one of a number of mechanisms. In one illustrated embodiment, two wheels with occluding
20 elements on their periphery cooperate in rotation to periodically pinch the tubular extrudate and thereby seal it. This mechanical compression system can be replaced by a variety of other mechanical or pneumatic compression systems to seal the tubular
25 extrudate at intervals.

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Alternatively, the system can include a flow control means for varying the pressure differential between the aqueous cell suspension and the polymeric solution during co-extrusion. For example, each of 5 the components supply means can include an infusion pump which is operated by a computer or other control element. In the normal operation, the infusion pumps are controlled to maintain a pressure differential between the aqueous cell suspension and the polymeric 10 solution, such that the polymer solvent is driven outward during coagulation. By periodically varying the pressure, the tubular extrudate can be collapsed at intervals to define individual cell compartments. This can be accomplished, for example, by reducing 15 the aqueous solution pressure. In some instances, it may be preferable to terminate the flow of the aqueous solution entirely and create a vacuum to ensure a complete seal between compartments.

20 Various other techniques can likewise be employed to interrupt the flow of the aqueous solution at intervals and thereby cause the tubular extrudate to collapse and form multiple compartments. For example, a retraction mechanism 25 can be incorporated into the extrusion head assembly for moving the inner bore relative to the outer bore, such that the flow of the aqueous solution is interrupted to define separate cell compartments at intervals.

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The systems disclosed herein can further include a quenchant bath for coagulating the polymeric solution following extrusion, and various mechanisms for drying the tubular extrudate as it emerges from the extrusion head, including blowers, or evacuation chambers. The extrusion head assembly can incorporate additional bores to provide multiple coatings or to deliver a quenchant fluid about the tubular extrudate. The system can also include a sedimentation chamber for the cell suspension, or an equivalent cell packing mechanism, to increase the cell density within the aqueous cell suspension.

The invention will next be described in connection with certain illustrated embodiments; however, it should be clear that various additions, subtractions or modifications can be made by those skilled in the art without departing from the spirit or scope of the invention.

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Brief Description of the Drawings

FIG. 1 is an overall schematic diagram of a system for encapsulating viable cells according to the invention;

FIG. 2 is a more detailed schematic diagram of an extrusion head assembly for use in the system of FIG. 1;

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FIG. 3 is a schematic diagram of an alternative extrusion head assembly for use in the system of FIG. 1;

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FIG. 4 is a schematic diagram of a mechanism for periodically sealing a tubular extrudate according to the invention to form a multi-compartment cell culturing vehicle;

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FIG. 5 is a schematic diagram of a mechanism for forming tethered cell capsules;

FIG. 6 is a graph showing dopamine release versus time for capsules containing dopamine secreting cells produced according to the present invention with three different solvent systems;

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FIG. 7 is graph showing dopamine release by PC12 cells under normal and potassium-stimulated conditions at various times following encapsulation according to the invention;

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FIG. 8A is a graph showing the release of catecholamines from encapsulated PC12 cells; and

FIG. 8B is a graph showing the release of 5 catecholamines from encapsulated chromaffin cells.

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Detailed Description

In FIG. 1, a system 10 is shown for producing a tubular extrudate 12 according to the present invention, including an extrusion head 14 having a first (innermost) bore 16, a second outer bore 18 and, optionally, a third (outermost) bore 20. The system 10 further includes a cell suspension supply 22 and an associated pump 24, a polymer solution supply 26 and an associated pump 28 and, optionally, a flush solution supply 30 with a pump 32. Additionally, the system can also, optionally, include a outer flowing quenchant supply 34 with an associated pump 36. All of the pump elements can be controlled manually or, preferably, by an automated controller (e.g., a microprocessor) 38. The system 10 can also include a quenchant bath 40, which would normally be disposed directly below the extrusion head 14 during operation. Alternatively, the system can include a blower 41 or the system can be employed within an evacuated or other reduced pressure chamber to aid in solvent removal.

When the system 10 is employed to shape the tubular extrudate into a multi-compartment cell capsule string, a sealing means can be employed. One such sealing element 42 is shown in FIG. 1, including two motorized wheels 44A and 44B which have a series of protuberances 46 which cooperate during rotation to periodically pinch and seal the tubular extrudate as it passes between the wheels 44A and 44B.

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Alternatively, a retraction means 48 can be employed to periodically retract the inner bore so as to interrupt the flow of the cell suspension. The effect of these retractions is to periodically seal the tubular extrudate and again form multiple compartments. In yet another alternative approach, the controller 38 can vary the pressure applied by pump 24 (and/or pump 28) to create periodic interruptions in the flow of the cell suspension.

10

In FIG. 2, the extrusion head 14 is shown in more detail, including an inner bore 16 for delivery of a cell suspension and an outer bore 18 for delivery of a polymeric solution. As the cell suspension and the polymeric solution are extruded through the common extrusion pore 19, the polymeric solution coagulates to form an outer coating about the cell suspension.

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In FIG. 3, an alternative extrusion head 14A is shown in more detail comprising an inner bore 16 for the delivery of the cell suspension, a second bore 18 (surrounding the inner bore) for delivery of the polymeric solution, and an outer most bore 20 for delivery of a flowing quenchant fluid, such as saline. In this embodiment, a smooth coating can be obtained by simultaneously extruding the cell suspension and polymeric solution through common pore 19 while applying a flowing quenchant fluid during the extrusion (e.g., from the outer most bore 20 in the extrusion head assembly 14A.)

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In FIG. 4, the sealing element 42 of FIG. 1 is shown in more detail. Motorized wheels 44A and 44B are mounted on opposite sides of the tubular extrudate 12, such that upon rotation protuberances 5 46 on the wheels periodically come in contact with the extrudate 12 to pinch and seal the extrudate 12 as it exits the extrusion head 14. The wheels 44A and 44B can be mechanically linked and operated by a conventional motor under the control of a controller, 10 such as shown in FIG. 1. The result of the periodic sealing of the extrudate 12 is a multi-compartment macrocapsule strand 50 having a polymeric membrane 52 surrounding an encapsulated cell solution 54 with individual cells 56 disposed therein. The individual 15 cell capsules are joined to each another by connective filaments 58 where the protuberances 46 of the sealing means 42 has pinched the extrudate 12.

Various polymers can be used to form the 20 membrane coatings of the present invention, including polymers derived from solutions which would otherwise be incompatible with the propagation of living cells. Because of the unique extrusion process disclosed in the present invention, solvents which 25 would otherwise be toxic are quickly driven away from the aqueous cell suspension during the membrane formation process, thereby permitting the use of many new and potentially useful polymeric materials. For example, polymeric membranes can be formed from 30 polyacrylates (including acrylic copolymers), polyvinylidienes, polyurethanes, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones, polyacrylonitriles, as well as derivatives, copolymers, and mixtures thereof.

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The solvent for the polymer solution will depend upon the particular polymer chosen for the membrane material. Suitable solvents include a wide variety of organic solvents, such as alcohols and 5 ketones, generally, as well as dimethylsulfoxide (DMSO), dimethylacetamide (DMA) and dimethylformimide (DMF), in particular. In general, water-miscible organic solvents are preferred.

10 The polymeric solution or "dope" can also include various additives, including surfactants to enhance the formation of porous channels, as well as antioxidants to sequester oxides that are formed during the coagulation process. Moreover, when the 15 cell capsules of the present invention are designed for implantation, materials, such as anti-inflammatory agents and cell growth factors, can also be incorporated into the polymeric membrane to reduce immune response or stimulate the cell culture, 20 respectively. Alternatively, these materials can be added to the multi-compartment cell capsule strands after formation by a post-coating or spraying process. For example, the strands can be immersed in a solution which contains an anti-inflammatory agent, 25 such as a corticoid, an angiogenic factor, or a growth factor following extrusion to post-coat the cell capsules.

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Post coating procedures can also be used to provide a protective barrier against immunogens and the like. For example, after formation, the cell capsule strands can be coated (e.g., by immersion, 5 spraying or applying a flowing fluid during extrusion) with a surface protecting material, such as polyethylene oxide or polypropylene oxide (e.g., having a molecular weight of about 10,000 Daltons or greater), to inhibit protein interactions with the 10 capsules.

Various techniques can also be employed to control the smoothness or roughness of the outer surface of the polymeric coating. In some instances, 15 a very smooth outer coating can be preferable to reduce scar tissue attachment and other immunoreactions during implantation. Such a smooth coating can be obtained by simultaneously immersing the tubular extrudate in a quenchant, such as a bath 20 of physiological saline, or by applying a flowing, quenchant fluid during the extrusion (e.g., from a third, concentric, outermost bore in an extrusion head assembly). Alternatively, in some applications a rough outer surface with larger pores may be 25 desired, for example, in instances where capillary ingrowth during implantation is desired, and such a rougher outer surface can be obtained by coagulation in air.

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Various cell lines can be encapsulated according to the present invention. As noted above, the multi-compartment cell culture strings are particularly useful for the constitutive delivery of neurotransmitters, such as dopamine, which is secreted by cells of the adrenal medulla, embryonic ventral mesencephalic tissue and neuroblastic cell lines. PC12 cells (an immortalized cell line derived from a rat pheocromocytoma) are particularly preferred in some applications because of their ability to secrete large amounts of dopamine over long periods of time. Other neurotransmitters include gamma aminobutyric acid (GABA), serotonin, acetylcholine, noradrenaline and other compounds necessary for normal nerve functions. A number of cell lines are known or can be isolated which secrete these neurotransmitters. Cells can also be employed which synthesize and secrete agonists, analogs, derivatives or fragments of neurotransmitters which are active, including, for example, cells which secrete bromocriptine, a dopamine agonist, and cells which secrete L-dopa, a dopamine precursor.

In other embodiments of the invention, the encapsulated cells can be chosen for their secretion of hormones, cytokines, nerve growth factors, angiogenesis factors, antibodies, blood coagulation factors, lymphokines, enzymes, and other therapeutic agents.

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The aqueous cell suspensions can further include various additives to protect the cells during the extrusion process or to stimulate their growth subsequently. Such additives can include, for
5 example a nutrient medium or growth factors which are incorporated into the aqueous suspension, as well as an anchorage substrate material to enhance cell attachment. The anchorage substrate material can be a proteinaceous material, such as collagen, laminin,
10 or polyamino acids. Alternatively, the cell suspension or the polymeric solution (or both) can include a foaming agent or a blowing agent which can distort the inner surface of the polymeric coating to increase the anchorage surface area of the tubular
15 interior.

The products of the present invention can take various forms, including simple tubular extrudates as well as multi-compartment cell capsule
20 strings. The shape of the multi-compartment strings can be tubular, resembling sausages, or nearly spherical, resembling strings of pearls. The maximum outer diameter of the strand with typically range from about 0.1 to about 1.0 millimeters. The
25 membrane wall thickness will typically range from about 10 to about 100 micrometers. The strand length of the strands will vary depending upon the particular application.

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The products can also take the form of "tethered" cell capsules, that is, one or more individual cell compartments corrected to a long polymeric tube or string. In FIG. 5, such a tethered cell capsule 51 is shown having a polymeric membrane 52 surrounding an encapsulated cell solution 54 with individual cells 56 disposed therein. The cell capsule 51 further includes a long polymeric filament 59 which can be formed by the same apparatus as described above in connection with FIG. 4 by interrupting the flow of the cell solution and constraining the polymeric solution to form a solid tether. The tether also can be post coated with a material (e.g., a polyurethane or the like) which imparts additional strength to the filament. Such tether cell capsules can find a variety of applications, particularly when implanted in a subject for constitutive delivery of active factors. In use, the cell capsule can be located as close to the target region (e.g., in the brain, peritoneal cavity or elsewhere) as desired while the other end of the tether can be fixed at a convenient anchor point or disposal in a readily accessible location for retrieval.

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The invention will next be described in connection with certain illustrative, non-limiting examples:

5

EXAMPLES

An extrusion system similar that illustrated in FIG. 1 was used, consisting of three electronically controlled programmable infusion pumps, a jet spinneret, two motor-controlled, coaxial wheel systems on the perimeter of which occluding polytetrafluoroethylene tubes were mounted, and an uptake system.

15 The macrocapsules were formed by injection of a polymeric solution into the outer tube of the spinneret. A coagulant, typically the encapsulated cells in their culture medium, was simultaneously injected in the spinneret inner tube. The
20 encapsulating membrane was formed by a dry-jet, wet spinning process, i.e., the fast stabilization of the polymer solution emerging from the spinneret nozzle by the internal quench medium coupled with further stabilization in a quench bath. The closure of the
25 fiber was performed by mechanically squeezing the forming hollow fiber with the coaxial wheel system prior to immersion in the quench bath. Near the spinneret head, the solvent concentration was sufficiently high to allow proper fusion of the fiber
30 wall. Following each round of encapsulation, pure solvent was flushed automatically through the lumen of the spinneret to avoid clogging of the nozzle.

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PC12 cells, an immortalized cell line derived from a rat pheocromocytoma which secretes large amounts of dopamine, were cultivated on collagen-coated tissue culture dishes in RPMI 1640 medium supplemented with 10% heat inactivated horse serum and 5% fetal calf serum. Dissociated bovine adrenal medullary cells, a non-dividing cell type which secretes dopamine, were maintained in DMEM medium supplemented with 5% fetal calf serum. Prior to encapsulation, the cells were harvested and loaded at a concentration of 1×10^5 cells/ml in a 3 ml syringe. A 15 percent vinylchloride-acrylonitrile copolymer solution in either dimethylsulfoxide (DMSO), dimethylformimide (DMF), or dimethylacetamide (DMAC) was loaded into a 5 ml glass syringe. Both solutions were then coextruded through the spinneret, and the capsules were collected in a physiologic saline solution. The capsules were rinsed and placed in individual wells containing the appropriate culture media.

Basal and potassium-evoked release of catecholamines was quantified under static incubation conditions by ion-pair reverse-phase high performance liquid chromatography (HPLC) equipped with electrochemical detection at 2 and 4 weeks. Morphological analysis, including light, scanning, and transmission electron microscopy, was performed on representative samples for each time period.

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All cell-loaded capsules released dopamine into the medium under basal conditions at all time periods. High potassium treatment increased dopamine release from both PC12 and adrenal medullary cells.

- 5 Dopamine output by PC12 cells, but not adrenal medullary cells, increased with time. The increase in dopamine release by the PC12 cell-loaded capsules over time is believed to be related to cell proliferation within the polymer capsule. No
- 10 significant difference in dopamine release could be observed from PC12-loaded capsules extruded with the three different solvent systems (DMSO, DMF, DMAC), which suggests that the encapsulation technique of the present invention may prevent cell damage
- 15 inflicted by solvents (FIG. 6). Due to the higher pressure of the inner bore system, the solvent was quickly driven toward the outside of the polymer capsule which prevented extended cell-solvent contact.

- 20 Morphological analysis revealed the presence of small clusters of PC12 cells randomly dispersed throughout the lumen of the capsule. At the electron microscope level, well-preserved PC12 cells, with their typical electron-dense secretory granules,
- 25 could be observed. Cell division within the capsule space was suggested by the presence of numerous mitotic figures. Although initially coextruded as a cell suspension, adrenal chromaffin cells formed packed aggregates one week after encapsulation.

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FIG. 7 shows the results of an in vitro assay in which PC12 cells were encapsulated according to the present invention and monitored for release dopamine at two and four weeks following

5 encapsulation. Dopamine levels were measured under both normal (controlled) conditions and also under a high potassium stimulation, which is known to induce depolarization of the cells and, consequently, to increase the secretion of dopamine in viable cells.

10 As can be seen from the graph, there was little activity at two weeks; however, at four weeks the encapsulated cells exhibited dopamine secretions not only under normal conditions but also exhibited a strong response to the potassium stimulation,

15 indicating that the cells were indeed viable in their encapsulated state.

FIG. 8A and 8B shows the results of further in vitro assays in which the secretions of PC12 cells

20 and chromaffin cells, respectively, were monitored four weeks after encapsulation according to the present invention. Again, the cells were stimulated by high potassium concentrations and the medium while the PC12 cells released only dopamine, the chromaffin

25 cells released a variety of catecholamines. The graft shows the levels of noradrenaline (NE), epinephrine (EPI), and dopamine (DA).

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Due to their fluid dynamics, the macrocapsules extruded in accordance with the present invention will allow the use of a wider range of polymer/solvent systems and can constitute a more efficient encapsulation technique. The results show that immortalized and differentiated dopamine-secreting cells will survive in macroencapsulation. The ability of these capsules to spontaneously release dopamine over time suggests that polymer encapsulation can provide an alternative to the transplantation of non-encapsulated or microencapsulated dopamine-secreting cells in the treatment of Parkinson's disease.

Claims

1. A method of encapsulating viable cells, the method comprising co-extruding an aqueous cell suspension and a polymeric solution through a common extrusion port to form a tubular extrudate having a polymeric outer coating which encapsulates said cell suspension.
2. The method of claim 1 wherein the method further comprises extruding the aqueous cell suspension and the polymeric solution through a common extrusion port having at least two concentric bores, such that said cell suspension is extruded through an inner bore and said polymeric solution is extruded through an outer bore.
3. The method of claim 1 wherein the method further includes sealing the ends of said tubular extrudate.
4. The method of claim 1 wherein the method further includes sealing the tubular extrudate at intervals to define separate cell compartments connected by polymeric links.
5. The method of claim 4 wherein the step of sealing the extrudate further comprises compressing the extrudate at intervals to define separate cell compartments.

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6. The method of claim 4 wherein the step of sealing the extrudate further comprises modifying the pressure under which the cell suspension or the polymeric solution is extruded, thereby collapsing
5 the tubular extrudate at intervals to define separate cell compartments.

7. The method of claim 4 wherein the step of sealing the extrudate further comprises impeding
10 the flow of the cell suspension at intervals to collapse the tubular extrudate and define separate cell compartments.

8. The method of claim 1 wherein the
15 polymeric coating is coagulated in air.

9. The method of claim 1 wherein the polymeric coating is coagulated in a reduced pressure chamber.
20

10. The method of claim 1 wherein the polymeric coating is coagulated in a quenchant bath.

11. The method of claim 1 wherein the
25 method further comprises extruding an aqueous suspension containing cells that secrete a biologically-active factor.

12. The method of claim 1 where in the
30 method further comprises extruding an aqueous suspension containing cells that secrete a neurotransmitter.

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13. The method of claim 1 wherein the method further comprises extruding an aqueous cell suspension which contains a nutrient medium.

5 14. The method of claim 1 wherein the method further comprises extruding an aqueous cell suspension which also contains an anchorage substrate.

10 15. The method of claim 1 wherein the method further includes extruding a polymeric solution which further comprises a water-miscible solvent component.

15 16. The method of claim 1 wherein the method further includes extruding a polymeric solution which further comprises a surfactant.

20 17. The method of claim 1 wherein the method further includes extruding a polymeric solution which further comprises an anti-inflammatory agent.

25 18. The method of claim 1 wherein the method further includes extruding a polymeric solution which further comprises an antioxidant.

30 19. The method of claim 1 wherein the method further comprises controlling the viscosity of said polymeric solution, such that upon coagulation said outer polymeric coating will form a semipermeable membrane about said cell suspension.

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20. The method of claim 1 wherein the method further comprises maintaining a pressure differential between the aqueous cell suspension and the polymeric solution during co-extrusion to impede solvent diffusion from said polymeric solution into said cell suspension.

21. The method of claim 1 wherein the method further comprises applying a protective barrier material to the outside of the polymeric coating.

22. A tubular extrudate prepared by the method of claim 1.

15

23. A cell culturing vehicle comprising a tubular, semipermeable, polymeric membrane encasing a cell culture, said tubular membrane being formed by extrusion.

20

24. The vehicle of claim 23 wherein the membrane is permeable to molecules having a molecular weight of about 150,000 or less.

25

25. The vehicle of claim 23 wherein the maximum outer diameter of the cell culture vehicle ranges from about 0.1 to about 1.0 millimeters.

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26. The vehicle of claim 23 wherein the membrane has a wall thickness ranging from about 10 to about 100 microns.

5

27. The vehicle of claim 23 wherein the polymeric membrane further comprises a polyacrylate material.

10

28. The vehicle of claim 23 wherein the polymeric membrane further comprises a surfactant.

29. The vehicle of claim 23 wherein the polymeric membrane further comprises an
15 anti-inflammatory agent.

30. The vehicle of claim 23 wherein the polymeric membrane further comprises an anti-oxidant.

20

31. The vehicle of claim 23 wherein the polymeric solution further comprises a water-miscible solvent.

32. The vehicle of claim 23 wherein the
25 extruded tubular membrane is sealed at intervals to define separate cell compartments connected by polymeric links.

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33. The vehicle of claim 23 wherein the extruded tubular membrane is sealed to define at least one cell compartment connected to a tethering filament.

5

34. The vehicle of claim 23 wherein the cell culture further comprises an aqueous cell suspension containing cells that secrete a biologically-active factor.

10

35. The vehicle of claim 34 wherein the aqueous cell suspension further comprises a nutrient medium.

15

36. The vehicle of claim 23 wherein the cell culture further comprises an anchorage substrate material.

37. The vehicle of claim 36 wherein the anchorage material comprises a collagen material.

38. The vehicle of claim 36 wherein the anchorage material comprises a laminin material.

39. The vehicle of claim 36 wherein the anchorage material comprises a polyamino acid.

40. The vehicle of claim 23 wherein the vehicle further comprises a protective barrier material which coats at least a portion of the outer surface of the polymeric membrane.

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41. The vehicle of claim 40 wherein the protective barrier material is an inhibitor of protein interactions.

5 42. The vehicle of claim 40 wherein the protective barrier material is selected from the group consisting of polyethylene oxides, polypropylene oxides, derivatives and mixtures thereof.

10

43. The vehicle of claim 23 wherein the cell culture further comprises a culture of cells capable of secreting a therapeutic factor.

15 44. A system for encapsulating viable cells, the system comprising:

an extrusion head assembly having at least a first inner bore and a second, concentric, outer bore;

20 cell suspension supply means for supplying an aqueous cell suspension to the inner bore of said extrusion head assembly; and

polymeric solution supply means for supplying a polymeric solution to the outer bore of
25 the extrusion head assembly, such that said cell suspension and said polymeric solution can be co-extruded to form a tubular extrudate having a polymeric outer coating which encapsulates said cell suspension.

30

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45. The system of claim 44 wherein the system further comprises flow control means for maintaining a pressure differential between the aqueous cell suspension and the polymeric solution
5 during co-extrusion.

46 The system of claim 44 wherein the flow control means comprises a computer adapted to control a first infusion pump in said cell suspension supply
10 means and a second infusion pump in said polymeric solution supply means.

47. The system of claim 44 wherein the system further comprises a quenchant bath for
15 coagulating said polymeric solution following extrusion.

48. The system of claim 44 wherein the system further comprises sealing means for sealing
20 the tubular extrudate at intervals to define separate cell compartments connected by polymeric links.

49. The system of claim 48 wherein the sealing means further comprises means for compressing
25 the extrudate at intervals during extrusion to define separate cell compartments.

50. The system of claim 44 wherein the sealing means further comprises means for modifying
30 the pressure under which the cell suspension or the polymeric solution is extruded, thereby collapsing the tubular extrudate at intervals to define separate compartments.

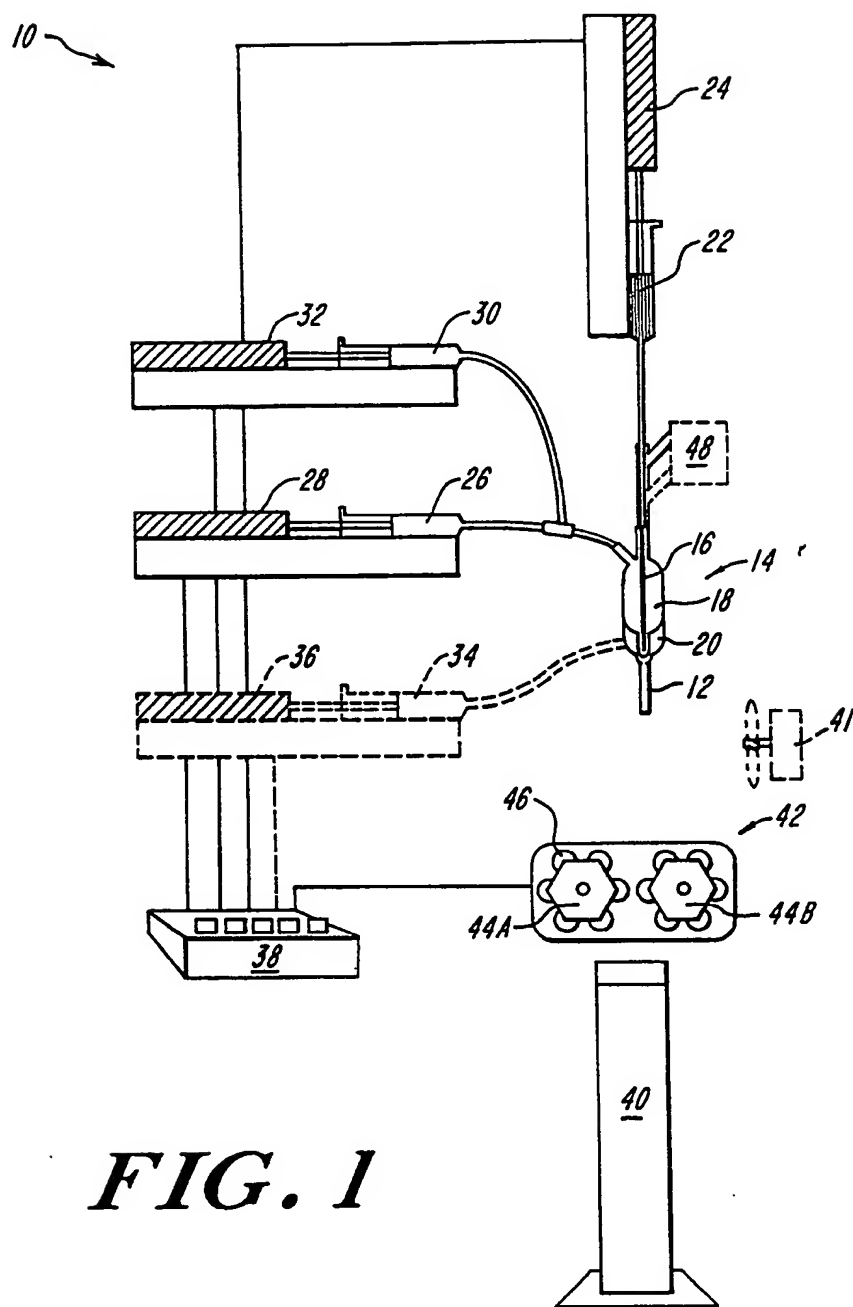
-34-

51. The system of claim 44 wherein the sealing means further comprises means for impeding the flow of the cell suspension at intervals to collapse the tubular extrudate and define separate cell compartments.

52. The system of claim 44 wherein the sealing means further comprises a retraction means for moving the inner bore of said extrusion head assembly relative to the outer bore to interrupt the flow of the tubular extrudate at intervals to define separate cell compartments.

53. The system of claim 44 wherein the extrusion head assembly includes a third, concentric, outer most bore for delivery of a quenchant fluid to coagulate said polymeric solution.

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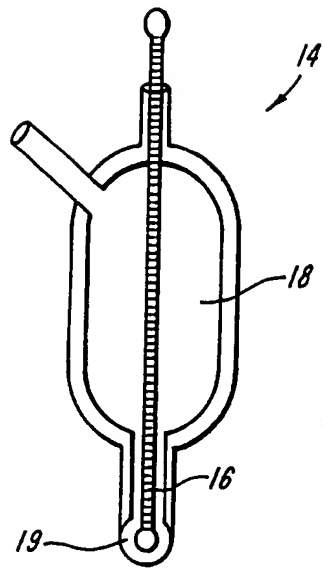


FIG. 2

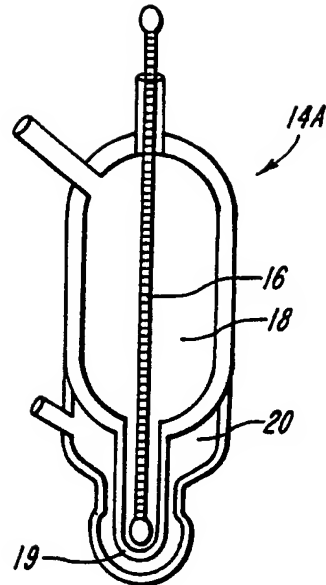


FIG. 3

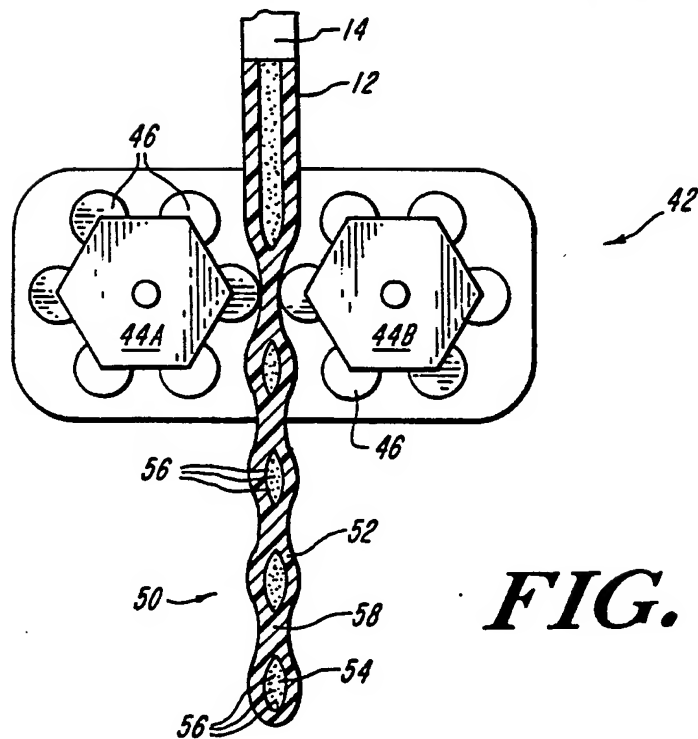
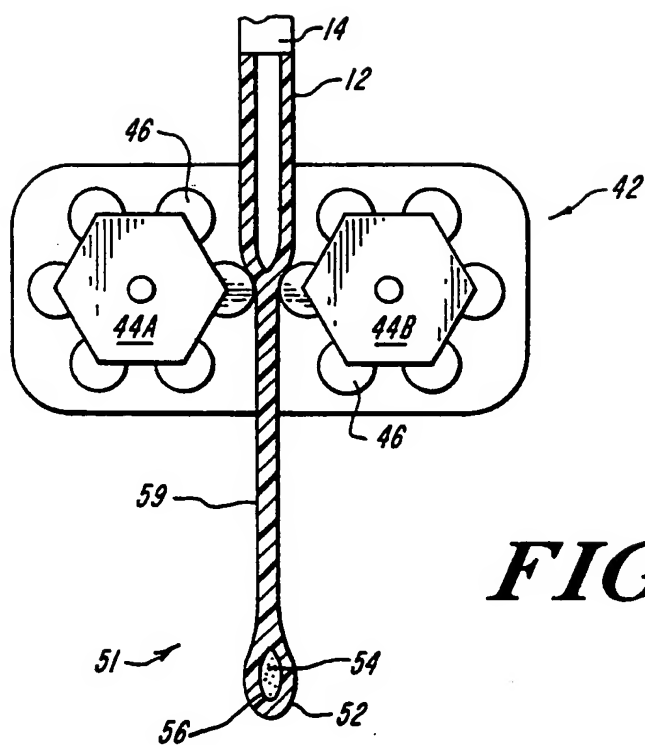
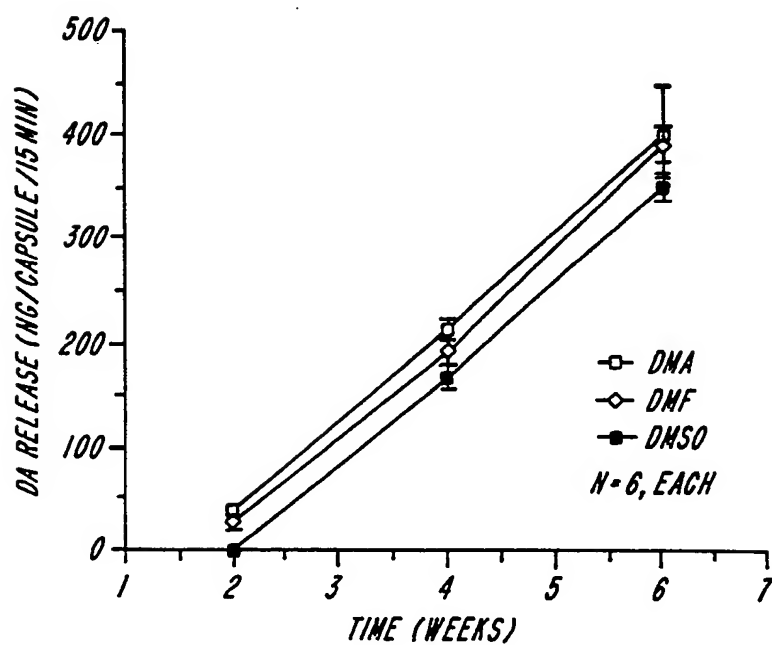
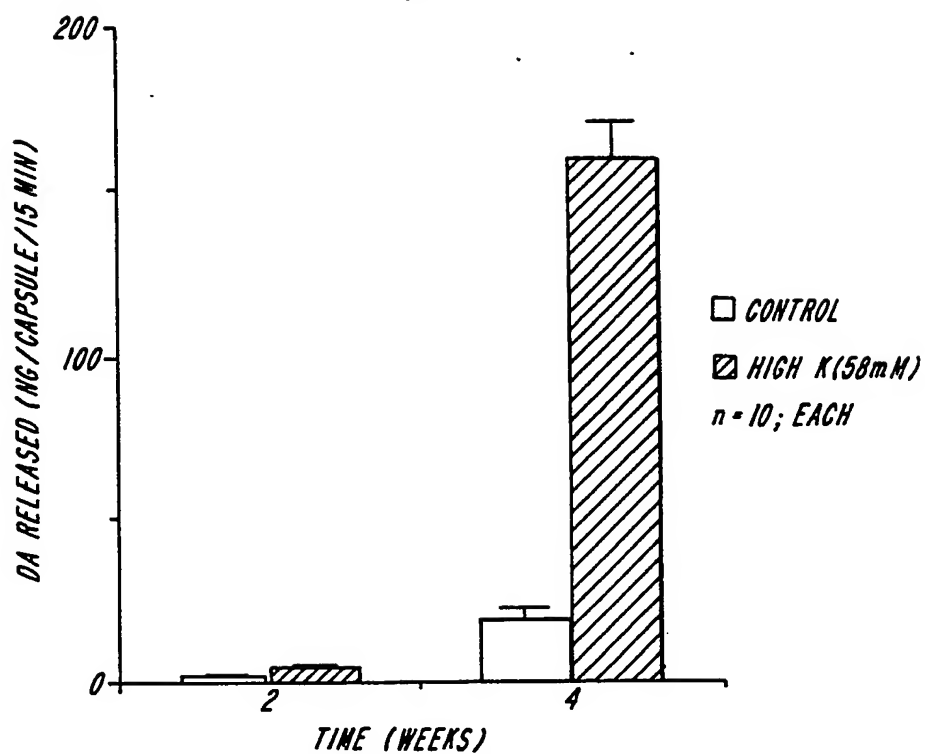
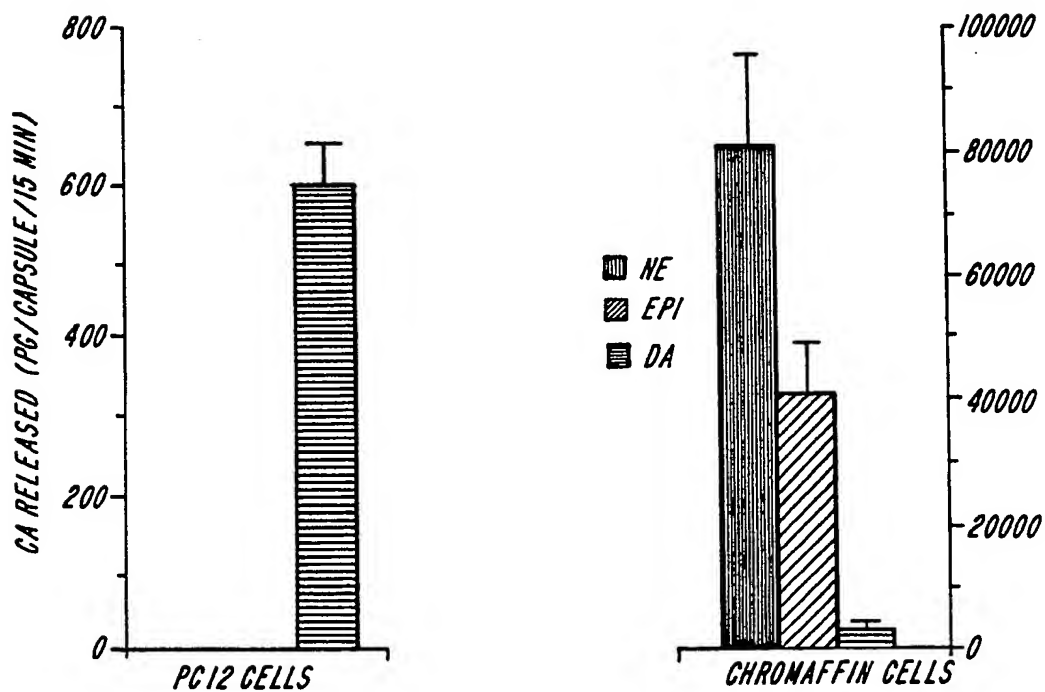


FIG. 4

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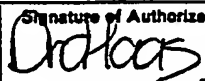
**FIG. 5****FIG. 6**

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**FIG. 7****FIG. 8A****FIG. 8B**

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/00157

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC ⁵ : A 61 K 9/50, C 12 N 11/04, B 01 J 13/04		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC ⁵	A 61 K, B 01 J	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	FR, A, 2599639 (RAMOT UNIVERSITY AUTHORITY) 11 December 1987 see page 3, lines 18-33; page 4, lines 27-35; page 5, lines 29-35 --	1-4, 8, 10, 14, 21-23, 25, 34, 36, 44, 45, 53
X	Patent Abstracts of Japan, vol. 11, no. 315 (C-451)(2762), 14 October 1987 & JP, A, 62100288 (HITACHI PLANT ENG & CONSTR. CO. LTD) 9 May 1987 see the abstract --	1
A	FR, A, 2336176 (MORISHITA JINTAN CO.) 22 July 1977 see page 1, lines 1-6; page 2, lines 27-39; page 5, lines 25-37 --	1-5
A	EP, A, 0188309 (CONNAUGHT LABORATORIES) 23 July 1986 ./.	1, 25, 27, 34, 40
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
18th April 1991	07.06.91	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	 Danielle van der Haas	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	see page 1, lines 19-22; page 3, lines 20-27 --	
A	WO, A, 8704367 (LTL ASSOCIATES) 30 July 1987 see page 5, lines 21-32; page 7, lines 4-12; page 9, lines 3-32; page 12, lines 14-27 --	1,3,4,27,34, 43
A	US, A, 4892538 (P.AEBISCHER et al.) 9 January 1990 see column 8, lines 18-46 (cited in the application) -----	1

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9100157

SA 44283

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 04/06/91
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A- 2599639	11-12-87	DE-A- 3718934	17-12-87
		GB-A, B 2192171	06-01-88
		JP-A- 63146792	18-06-88
FR-A- 2336176	22-07-77	JP-A- 52148635	10-12-77
		JP-A- 52148663	10-12-77
		JP-C- 924406	22-09-78
		JP-A- 52078775	02-07-77
		JP-B- 53001067	14-01-78
		DE-A, B, C 2658587	07-07-77
		GB-A- 1538510	17-01-79
		NL-A- 7614193	28-06-77
		US-A- 4426337	17-01-84
		US-A- 4251195	17-02-81
EP-A- 0188309	23-07-86	CA-A- 1258429	15-08-89
		JP-A- 61209586	17-09-86
WO-A- 8704367	30-07-87	AU-A- 6940087	14-08-87
		EP-A- 0259365	16-03-88
US-A- 4892538	09-01-90	AU-A- 2718488	14-06-89
		EP-A- 0388428	26-09-90
		WO-A- 8904655	01-06-89